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TITLE: Fusion proteins of novel CTLA4/CD28 ligands and uses therefore

Drawing Description Text (3):

FIGS. 2A-C are graphs of log fluorescence intensity of cell surface expression of B7-1 on splenic B cells activated with surface immunoglobulin (slg) crosslinking. The total (panel a), B7-1 positive (B7-1.sup.+, panel b) and B7-1.sup.- negative (B7-1-, panel c) activated B cells were stained with anti-B7-1 monoclonal antibody (133) and fluoroscein isothiocyanate (FITC) labeled goat anti-mouse immunoglobulin and analyzed by flow cytometry.

Drawing Description Text (13):

FIG. 12 is a graphic representation of the inhibition by mAbs and recombinant proteins of the proliferation of CD28+ T cells, as assessed by .sup.3 H-thymidine incorporation and IL-2 secretion, to stimulation by PMA and COS cells transfected with vector alone (vector), or with a vector expressing B7-1 (B7-1) or B7-2 (B7-2). Inhibition studies were performed with the addition of either no antibody (no mAb), anti-B7 mab 133 (133), anti-B7 mAb BB-1 (BB1), anti-B5 mAb (B5), Fab fragment of anti-CD28 (CD28 Fab), CTLA4Ig (CTLA4Ig), or Ig control protein (control Ig) to the PMA stimulated COS cell admixed CD28.sup.+ T cells.

Drawing Description Text (18):

FIG. 17 depicts flow cytometric profiles of cells stained with an anti-hB7-2 monoclonal antibody, HA3.1F9. Cells stained with the antibody were CHO cells transfected to express human B7-2 (CHO-hB7.2), NIH 3T3 cells transfected to express human B7-2 (3T3-hB7.2) and control transfected NIH 3T3 cells (3T3-neo). The anti-hB7.2 antibody B70 was used as a positive control.

Drawing Description Text (19):

FIG. 18 depicts flow cytometric profiles of cells stained with an anti-hB7-2 monoclonal antibody, HA5.2B7. Cells stained with the antibody were CHO cells transfected to express human B7-2 (CHO-hB7.2), NIH 3T3 cells transfected to express human B7-2 (3T3-hB7.2) and control transfected NIH 3T3 cells (3T3-neo). The anti-hB7.2 antibody B70 was used as a positive control.

Drawing Description Text (20):

FIG. 19 depicts flow cytometric profiles of cells stained with an anti-hB7-2 monoclonal antibody, HF2.3D1. Cells stained with the antibody were CHO cells transfected to express human B7-2 (CHO-hB7.2), NIH 3T3 cells transfected to express human B7-2 (3T3-hB7.2) and control transfected NIH 3T3 cells (3T3-neo). The anti-hB7.2 antibody B70 was used as a positive control.

Detailed Description Text (3):

The B lymphocyte antigen B7-2 is expressed by human B cells at about 24 hours following stimulation with either anti-immunoglobulin or anti-MHC class II monoclonal antibody. The B7-2 antigen induces detectable IL-2 secretion and T cell proliferation. At about 48 to 72 hours post activation, human B cells express both B7-1 and a third CTLA4 counter-receptor, B7-3, identified by a monoclonal antibody BB-1, which also binds B7-1 (Yokochi, T., et al. (1982) J. Immunol. 128, 823-827). The B7-3 antigen is also expressed on B7-1 negative activated B cells and can costimulate T cell proliferation without detectable IL-2 production, indicating that the B7-1 and B7-3 molecules are distinct. B7-3 is expressed on a wide variety of cells including activated B cells, activated monocytes, dendritic cells, Langerhan

cells and keratinocytes. At 72 hours post B cell activation, the expression of B7-1 and B7-3 begins to decline. The presence of these costimulatory molecules on the surface of activated B lymphocytes indicates that T cell costimulation is regulated, in part, by the temporal expression of these molecules following B cell activation.

Detailed Description Text (18):

Transfected cells which express peptides having an activity of one or more B lymphocyte antigens (e.g., B7-2, B7-3) on the surface of the cell are also within the scope of this invention. In one embodiment, a host cell such as a COS cell is transfected with an expression vector directing the expression of a peptide having B7-2 activity on the surface of the cell. Such a transfected host cell can be used in methods of identifying molecules which inhibit binding of B7-2 to its counter-receptor on T cells or which interfere with intracellular signaling of costimulation to T cells in response to B7-2 interaction. In another embodiment, a tumor cell such as a sarcoma, a melanoma, a leukemia, a lymphoma, a carcinoma or a neuroblastoma is transfected with an expression vector directing the expression of at least one peptide having the activity of a novel B lymphocyte antigen on the surface of the tumor cell. In some instances, it may be beneficial to transfect a tumor cell to coexpress major histocompatibility complex (MHC) proteins, for example MHC class II .alpha. and .beta. chain proteins or an MHC class I .alpha. chain protein, and, if necessary, a .beta.2 microglobulin protein. Such transfected tumor cells can be used to induce tumor immunity in a subject. These and other embodiments are described in further detail herein.

Detailed Description Text (29):

B7-3 and can alternatively serve as a source of the mRNA for construction of a cDNA library. For example, tumor cells isolated from patients with non-Hodgkins lymphoma express B7-1 mRNA. B cells from nodular, poorly differentiated lymphoma (NPD), diffuse large cell lymphoma (LCL) and Burkitt's lymphoma cell lines are also suitable sources of human B7-1 mRNA and, potentially B7-2 and B7-3 mRNA. Myelomas generally express B7-2, but not B7-1 mRNA, and, thus can provide a source of B7-2 mRNA. The Burkitt's lymphoma cell line Raji is one source of B lymphocyte antigen mRNA. Preferably, B7-2 mRNA is obtained from a population of both resting and activated normal human B cells. Activated B cells can be obtained by stimulation over a broad spectrum of time (e.g., from minutes to days) with, for example, an anti-immunoglobulin antibody or an anti-MCH class II antibody.

Detailed Description Text (37):

According to one embodiment, plasmid DNA is introduced into a simian COS cell line (Gluzman, Cell 23:175 (1981)) by known methods of transfection (e.g., DEAE-Dextran) and allowed to replicate and express the cDNA inserts. The transfectants expressing B7-1 antigen are depleted with an anti-B7-1 monoclonal antibody (e.g., 133 and B1.1) and anti-murine IgG and IgM coated immunomagnetic beads. Transfectants expressing human B7-2 antigen can be positively selected by reacting the transfectants with the fusion proteins CTLA4Ig and CD28Ig, followed by panning with anti-human Ig antibody coated plates. Although human CTLA4Ig and CD28Ig fusion proteins were used in the examples described herein, given the cross-species reactivity between B7-1 and, for example murine B7-1, it can be expected that other fusion proteins reactive with another cross-reactive species could be used. After panning, episomal DNA is recovered from the panned cells and transformed into a competent bacterial host, preferably E. coli. Plasmid DNA is subsequently reintroduced into COS cells and the cycle of expression and panning repeated at least two times. After the final cycle, plasmid DNA is prepared from individual colonies, transfected into COS cells and analyzed for expression of novel B lymphocyte antigens by indirect immunofluorescence with, for example, CTLA4Ig and CD28Ig.

Detailed Description Text (85):

The polyclonal or monoclonal antibodies of the current invention, such as an antibody specifically reactive with a recombinant or synthetic peptide having B7-2 activity or B7-3 activity can also be used to isolate the native B lymphocyte antigen from cells. For example, antibodies reactive with the peptide can be used to isolate the naturally-occurring or native form of B7-2 from activated B lymphocytes by immunoaffinity chromatography. In addition, the native form of B7-3 can be isolated from B cells by immunoaffinity chromatography with monoclonal antibody BB-1.

Detailed Description Text (92):

protein, or peptide fragment thereof, to identify and isolate packages that express an antibody having specificity for the B lymphocyte antigen. Nucleic acid encoding the selected antibody can be recovered from the display package (e.g., from the phage genome) and subcloned into other expression vectors by standard recombinant DNA techniques. Furthermore, a therapeutically active amount of one or more peptides having B7-2 activity and or B7-1 activity can be used in conjunction with other immunomodulating reagents to influence immune responses. Examples of other immunomodulating reagents include blocking antibodies, e.g., against CD28 or CTLA4, against other T cell markers or against cytokines, fusion proteins, e.g., CTLA4Ig, or immunosuppressive drugs, e.g., cyclosporine A or FK506.

Detailed Description Text (97):

Downregulating or preventing one or more B lymphocyte antigen functions, e.g., preventing high level lymphokine synthesis by activated T cells, will be useful in situations of tissue, skin and organ transplantation and in graft-versus-host disease (GVHD). For example, blockage of T cell function should result in reduced tissue destruction in tissue transplantation. Typically, in tissue transplants, rejection of the transplant is initiated through its recognition as foreign by T cells, followed by an immune reaction that destroys the transplant. The administration of a molecule which inhibits or blocks interaction of a B7 lymphocyte antigen with its natural ligand(s) on immune cells (such as a soluble, monomeric form of a peptide having B7-2 activity alone or in conjunction with a monomeric form of a peptide having an activity of another B lymphocyte antigen (e.g., B7-1, B7-3) or blocking antibody), prior to transplantation can lead to the binding of the molecule to the natural ligand(s) on the immune cells without transmitting the corresponding costimulatory signal. Blocking B lymphocyte antigen function in this manner prevents cytokine synthesis by immune cells, such as T cells, and thus acts as an immunosuppressant. Moreover, the lack of costimulation may also be sufficient to anergize the T cells, thereby inducing tolerance in a subject. Induction of long-term tolerance by B lymphocyte antigen-blocking reagents may avoid the necessity of repeated administration of these blocking reagents. To achieve sufficient immunosuppression or tolerance in a subject, it may also be necessary to block the function of a combination of B lymphocyte antigens. For example, it may be desirable to block the function of B7-2 and B7-1, B7-2 and B7-3, B7-1 and B7-3 or B7-2, B7-1 and B7-3 by administering a soluble form of a combination of peptides having an activity of each of these antigens or a blocking antibody (separately or together in a single composition) prior to transplantation. Alternatively, inhibitory forms of B lymphocyte antigens can be used with other suppressive agents such as blocking antibodies against other T cell markers or against cytokines, other fusion proteins, e.g., CTLA4Ig, or immunosuppressive drugs.

Detailed Description Text (100):

The IgE antibody response in atopic allergy is highly T cell dependent and, thus, inhibition of B lymphocyte antigen induced T cell activation may be useful therapeutically in the treatment of allergy and allergic reactions. An inhibitory form of B7-2 protein, such as a peptide having B7-2 activity alone or in combination with a peptide having the activity of another B lymphocyte antigen, such as B7-1, can be administered to an allergic subject to inhibit T cell mediated allergic responses in the subject. Inhibition of B lymphocyte antigen costimulation of T cells may be accompanied by exposure to allergen in conjunction with appropriate MHC molecules. Allergic reactions may be systemic or local in nature, depending on the route of entry of the allergen and the pattern of deposition of IgE on mast cells or basophils. Thus, it may be necessary to inhibit T cell mediated allergic responses locally or systemically by proper administration of an inhibitory form of B7-2 protein.

Detailed Description Text (101):

Inhibition of T cell activation through blockage of B lymphocyte antigen function may also be important therapeutically in viral infections of T cells. For example, in the acquired immune deficiency syndrome (AIDS), viral replication is stimulated by T cell activation. Blocking B7-2 function could lead to a lower level of viral replication and thereby ameliorate the course of AIDS. In addition, it may also be necessary to block the function of a combination of B lymphocyte antigens i.e.,

B7-1, B7-2 and B7-3. Surprisingly, HTLV-I infected T cells express B7-1 and B7-2. This expression may be important in the growth of HTLV-I infected T cells and the blockage of B7-1 function together with the function of B7-2 and/or B7-3 may slow the growth of HTLV-I induced leukemias. Alternatively, stimulation of viral replication by T cell activation may be induced by contact with a stimulatory form of B7-2 protein, for such purposes as generating retroviruses (e.g., various HIV isolates) in sufficient quantities for isolation and use.

Detailed Description Text (106):

In another application, upregulation or enhancement of B lymphocyte antigen function may be useful in the induction of tumor immunity. Tumor cells (e.g., sarcoma, melanoma, lymphoma, leukemia, neuroblastoma, carcinoma) transfected with a nucleic acid encoding at least one peptide having the activity of a B lymphocyte antigen, such as B7-2, can be administered to a subject to overcome tumor-specific tolerance in the subject. If desired, the tumor cell can be transfected to express a combination of peptides having the activity of a number of B lymphocyte antigens (e.g., B7-1, B7-2, B7-3). For example, tumor cells obtained from a patient can be transfected ex vivo with an expression vector directing the expression of a peptide having B7-2 activity alone, or in conjunction with a peptide having B7-1 activity and/or B7-3 activity. The transfected tumor cells are returned to the patient to result in expression of the peptides on the surface of the transfected cell. Alternatively, gene therapy techniques can be used to target a tumor cell for transfection in vivo.

Detailed Description Text (136):

Splenic B cells were cultured at 2.times.10.sup.6 cells/ml in complete culture media, {RPMI 1640 with 10% heat inactivated fetal calf serum (FCS), 2 mM glutamine, 1 mM sodium pyruvate, penicillin (100 units/ml), streptomycin sulfate (100 .mu.g/ml) and gentamycin sulfate (5 .mu.g/ml)}, in tissue culture flasks and were activated by crosslinking of slg with affinity purified rabbit anti-human IgM coupled to Affi-Gel 702 beads (Bio-Rad, Richmond, Calif.) (Boyd, A. W., et al., (1985) J. Immunol. 134,1516) or by crosslinking of MHC class II with 9-49 antibody coupled to Affi-Gel 702 beads. B cells activated for 72 hours, were used as total activated B cell populations or were indirectly stained with anti-B7 (B 1.1) monoclonal antibody and fluorescein isothiocyanate (FITC) labeled goat anti-mouse immunoglobulin (Fisher, Pittsburgh, PA), and fractionated into B7-1+ and B7-1- populations by flow cytometric cell sorting (EPICS Elite flow cytometer, Coulter).

Detailed Description Text (138):

For surface phenotype analysis populations of B cells activated by either sIg or MHC class II crosslinking for 6, 12, 24, 48, 72 and 96 hours were stained with either anti-B7 (133), BB-1 monoclonal antibodies, control IgM antibody, CTLA4Ig or control-Ig. Cell suspensions were stained by two step indirect membrane staining with 10 .mu.g/ml of primary monoclonal antibody followed by the appropriate secondary reagents. Specifically, immunoreactivity with anti-B7 (133) and BB-1 monoclonal antibodies was studied by indirect staining using goat anti-mouse Ig or immunoglobulin FITC (Fisher) as secondary reagent and immunoreactivity with fusion proteins was studied using biotinylated CTLA4Ig or biotinylated control-Ig and streptavidin-phycoerythrin as secondary reagent. PBS containing 10% AB serum was used as diluent and wash media. Cells were fixed with 0.1% paraformaldehyde and analyzed on a flow cytometer (EPICS Elite Coulter).

Detailed Description Text (145):

Since crosslinking surface Ig induces human resting B cells to express B7-1 maximally (50-80%) at 72 hours, the ability of activated human B lymphocytes to induce submitogenically activated T cells to proliferate and secrete IL-2 was determined. FIG. 1 depicts the costimulatory response of human splenic CD28.sup.+ T cells, submitogenically activated with anti-CD3 monoclonal antibody, to either B7 (B7-1) transfected CHO cells (CHO-B7) or syngeneic splenic B cells activated with anti-Ig for 72 hours. .sup.3 H-Thymidine incorporation was assessed for the last 15 hours of a 72 hours culture. IL-2 was assessed by ELISA in supernatants after 24 hours of culture (Detection limits of the assay: 31-2000 pg/ml). FIG. 1 is representative of seventeen experiments.

Detailed Description Text (146):

Submitogenically activated CD28+ T cells proliferated and secreted high levels of IL-2 in response to B7-1 costimulation provided by CHO-B7 (FIG. 1, panel a). Both proliferation and IL-2 secretion were totally inhibited by blocking the B7-1 molecule on CHO cells with either anti-B7-1 monoclonal antibody or by a fusion protein for its high affinity receptor, CTLA4. Similarly, proliferation and IL-2 secretion were abrogated by blocking B7-1 signalling via CD28 with Fab anti-CD28 monoclonal antibody. Control monoclonal antibody or control fusion protein had no effect. Nearly identical costimulation of proliferation and IL-2 secretion was provided by splenic B cells activated with anti-Ig for 72 hours (panel b). Though anti-B7-1 monoclonal antibody could completely abrogate both proliferation and IL-2 secretion delivered by CHO-B7, anti-B7-1 monoclonal antibody consistently inhibited proliferation induced by activated B cells by only 50% whereas IL-2 secretion was totally inhibited. In contrast to the partial blockage of proliferation induced by anti-B7-1 monoclonal antibody, both CTLA4Ig and Fab anti-CD28 monoclonal antibody completely blocked proliferation and IL-2 secretion. These results are consistent with the hypothesis that activated human B cells express one or more additional CTLA4/CD28 ligands which can induce T cell proliferation and IL-2 secretion.

Detailed Description Text (149):

In light of the above observations, whether other CTLA4 binding counter-receptors were expressed on activated B cells was determined. To this end, human splenic B cells were activated for 72 hours with anti-Ig and then stained with an anti-B7-1 monoclonal antibody (B 1.1) which does not inhibit B7-1 mediated costimulation. Fluorescein isothiocyanate (FITC) and mAb B 1.1 were used with flow cytometric cell sorting to isolate B7-1.sup.+ and B7-1.sup.- fractions. The resulting post-sort positive population was 99% B7-1.sup.+ and the post-sort negative population was 98% B7-1.sup.- (FIG. 2).

Detailed Description Text (150):

To examine the costimulatory potential of each population, human splenic CD28+ T cells were submitogenically stimulated with anti-CD3 monoclonal antibody in the presence of irradiated B7-1+ or B7-1- anti-Ig activated (72 hours) splenic B cells. .sup.3 H-Thymidine incorporation was assessed for the last 15 hours of a 72 hours culture. IL-2 was assessed by ELISA in supernatants after 24 hours of culture (Detection limits of the assay: 31-2000 pg/ml). The results of FIG. 3 are representative of ten experiments. B7-1+ B cells induced anti-CD3 activated T cells to proliferate and secrete IL-2 (FIG. 3a) but not IL-4. As was observed with the unfractionated activated B cell population, anti-B7-1 monoclonal antibody (133) inhibited proliferation only 50% but consistently abrogated IL-2 secretion. As above, CTLA4Ig binding or blockade of CD28 with Fab anti-CD28 monoclonal antibody completely inhibited both proliferation and IL-2 secretion. Control monoclonal antibody and control-Ig were not inhibitory. In an attempt to identify other potential CTLA4/CD28 binding costimulatory ligand(s) which might account for the residual, non-B7 mediated proliferation delivered by B7+ B cells, the effect of BB-1 monoclonal antibody on proliferation and IL-2 secretion was examined. As seen, BB-1 monoclonal antibody completely inhibited both proliferation and IL-2 secretion (FIG. 3a). FIG. 3b displays the costimulatory potential of B7-1- activated human splenic B cells. Irradiated B7-1- activated (72 hr) B cells could also deliver a significant costimulatory signal to submitogenically activated CD4+ lymphocytes. This costimulation was not accompanied by detectable IL-2 (FIG. 3b) or IL-4 accumulation and anti-B7-1 monoclonal antibody did not inhibit proliferation. However, CTLA4Ig, Fab anti-CD28 monoclonal antibody, and BB-1 monoclonal antibody all completely inhibited proliferation.

Detailed Description Text (151):

Phenotypic analysis of the B7-1+ and B7-1- activated splenic B cells confirmed the above functional results. FIG. 4 shows the cell surface expression of B7-1, B7-2 and B7-3 on fractionated B7-1.sup.+ and B7-1.sup.- activated B cell. As seen in FIG. 4, B7-1+ activated splenic B cells stained with anti-B7-1 (133) monoclonal antibody, BB-1 monoclonal antibody, and bound CTLA4-Ig. In contrast, B7- activated splenic B cells did not stain with anti-B7-1 (133) monoclonal antibody but did stain with BB-1 monoclonal antibody and CTLA4Ig. These phenotypic and functional results demonstrate that both B7-1+ and B7-1-activated (72 hours) human B lymphocytes express CTLA4 binding counter-receptor(s) which: 1) can induce submitogenically activated T cells to proliferate without detectable IL-2 secretion; and 2) are identified by the BB-1

monoclonal antibody but not anti-B7-1 monoclonal antibody. Thus, these CTLA4/CD28 ligands can be distinguished on the basis of their temporal expression after B cell activation and their reactivity with CTLA4Ig and anti-B7 monoclonal antibodies. The results of FIG. 4 are representative of five experiments.

Detailed Description Text (155):

A series of experiments was conducted to determine whether the temporal expression of CTLA4 binding counter-receptors differentially correlated with their ability to costimulate T cell proliferation and/or IL-2 secretion. Human splenic CD28+ T cells submitogenically stimulated with anti-CD3 were cultured for 72 hours in the presence of irradiated human splenic B cells that had been previously activated in vitro by slg crosslinking for 24, 48, or 72 hours. IL-2 secretion was assessed by ELISA in supernatants after 24 hours and T cell proliferation as assessed by ³H-thymidine incorporation for the last 15 hours of a 72 hour culture. The results of FIG. 7 are representative of 5 experiments. As seen in FIG. 7a, 24 hour activated B cells provided a costimulatory signal which was accompanied by modest levels of IL-2 production, although the magnitude of proliferation was significantly less than observed with 48 and 72 hours activated human B cells (note differences in scale for ³H-Thymidine incorporation). Neither proliferation nor IL-2 accumulation was inhibited by anti-B7-1 (133) or BB-1. In contrast, with CTLA4Ig and anti-CD28 Fab monoclonal antibody totally abrogated proliferation and IL-2 accumulation. B cells activated for 48 hours, provided costimulation which resulted in nearly maximal proliferation and IL-2 secretion (FIG. 7b). Here, anti-B7-1 (133) monoclonal antibody, inhibited proliferation approximately 50% but totally blocked IL-2 accumulation. BB-1 monoclonal antibody totally inhibited both proliferation and IL-2 secretion. As above, CTLA4Ig and Fab anti-CD28 also totally blocked proliferation and IL-2 production. Finally, 72 hour activated B cells induced T cell response identical to that induced by 48 hour activated B cells. Similar results are observed if the submitogenic signal is delivered by phorbol myristic acid (PMA) and if the human splenic B cells are activated by MHC class II rather than Ig crosslinking. These results indicate that there are three CTLA4 binding molecules that are temporarily expressed on activated B cells and each can induce submitogenically stimulated T cells to proliferate. Two of these molecules, the early CTLA4 binding counter-receptor (B7-2) and B7-1 (133) induce IL-2 production whereas B7-3 induces proliferation without detectable IL-2 production.

Detailed Description Text (156):

Previous studies provided conflicting evidence whether the anti-B7 monoclonal antibody, 133 and monoclonal antibody BB-1 identified the same molecule (Freedman, A. S. et al. (1987) Immunol. 137, 3260-3267; Yokochi, T., et al. (1982) J. Immunol. 128, 823-827; Freeman, G. J., et al. (1989) J. Immunol. 143, 2714-2722.). Although both monoclonal antibodies identified molecules expressed 48 hours following human B-cell activation, several reports suggested that B7 (B7-1) and the molecule identified by monoclonal antibody BB-1 were distinct since they were differentially expressed on cell lines and B cell neoplasms (Freedman, A. S. et al. (1987) Immunol. 137, 3260-3267; Yokochi, T., et al. (1982) J. Immunol. 128, 823-827; Freeman, G. J., et al. (1989) J. Immunol. 143, 2714-2722; Clark, E and Yokochi, T. (1984) Leukocyte Typing, 1st International References Workshop. 339-346; Clark, E., et al. (1984) Leukocyte Typing, 1st International References Workshop. 740). In addition, immunoprecipitation and Western Blotting with these IgM monoclonal antibodies suggested that they identified different molecules (Clark, E and Yokochi, T. (1984) Leukocyte Typing, 1st International References Workshop. 339-346; Clark, E., et al. (1984) Leukocyte Typing, 1st International References Workshop. 740). The original anti-B7 monoclonal antibody, 133, was generated by immunization with anti-immunoglobulin activated human B lymphocytes whereas the BB-1 monoclonal antibody was generated by immunization with a baboon cell line. Thus, the BB-1 monoclonal antibody must identify an epitope on human cells that is conserved between baboons and humans.

Detailed Description Text (157):

Following the molecular cloning and expression of the human B7 gene (B7-1), B7 transfected COS cells were found to be identically stained with the anti-B7 (133) and BB-1 monoclonal antibodies and that they both precipitated the identical broad molecular band (44-54 kD) strongly suggesting that they identified the same molecule (Freeman, G. J., et al. (1989) J. Immunol. 143, 2714-2722). This observation was

unexpected since the gene encoding the molecule identified by the BB-1 monoclonal antibody had been previously mapped to chromosome 12 (Katz, F. E., et al. (1985) Eur. J. Immunol. 103-6), whereas the B7 gene was located by two groups on chromosome 3 (Freeman, G. J., et al. (1992) Blood. 79, 489-494; Selvakumar, A., et al. (1992) Immunogenetics 36, 175-181.). Subsequently, additional discrepancies between the phenotypic expression of B7 (B7-1) and the molecule identified by the BB-1 monoclonal antibody were noted. BB-1 monoclonal antibody stained thymic epithelial cells (Turka, L. A., et al. (1991) J. Immunol. 146, 1428-36; Munro, J. M., et al. Blood submitted.) and keratinocytes (Nickoloff, B., et al (1993) Am. J. Pathol. 142, 1029-1040; Augustin, M., et al. (1993) J. Invest. Dermatol. 100, 275-281.) whereas anti-B7 did not. Recently, Nickoloff et al. (1993) Am. J. Pathol. 142, 1029-1040, reported discordant expression of the molecule identified by the BB-1 monoclonal antibody and B7 on keratinocytes using a BB-1 and anti-B7 (B 1.1 and 133) monoclonal antibodies. Nickoloff et al. also demonstrated that these BB-1 positive cells did not express B7 mRNA yet bound CD28 transfected COS cells providing further support for the existence of a distinct protein which binds monoclonal antibody BB-1.

Detailed Description Text (158):

The present findings confirm that there is an additional CTLA4 counter-receptor identified by the BB-1 monoclonal antibody, B7-3, and that this protein appears to be functionally distinct from B7-1 (133). Although the expression of B7-1 and B7-3 following B cell activation appears to be concordant on B7 positive B cells, these studies demonstrate that the B7-3 molecule is also expressed on B7 negative activated B cells. More importantly, the B7-3 molecule appears to be capable of inducing T

Detailed Description Text (159):

cell proliferation without detectable IL-2 or IL-4 production. This result is similar to the previous observation that ICAM-1 could costimulate T cell proliferation without detectable IL-2 or IL-4 production (Boussiotis, V., et al J. Exp. Med. (accepted for publication)). These data indicate that the BB-1 monoclonal antibody recognizes an epitope on the B7-1 protein and that this epitope is also found on a distinct B7-3 protein, which also has costimulatory function. Phenotypic and blocking studies demonstrate that the BB-1 monoclonal antibody could detect one (on B7 negative cells) or both (on B7 positive cells) of these proteins. In contrast, the anti-B7 monoclonal antibodies, 133 and B 1.1 detect only the B7-1 protein. Taken together, these results suggest that by 48 hours post B-cell activation by crosslinking of surface immunoglobulin or MHC class II, B cells express at least two distinct CTLA4 binding counter-receptors, one identified by both anti-B7 and BB-1 monoclonal antibodies and the other identified only by BB-1 monoclonal antibody.

Detailed Description Text (160):

The B7-2 antigen is not detectable on activated B cells after 12 hours, but by 24 hours it is strongly expressed and functional. This molecule appears to signal via CD28 since proliferation and IL-2 production are completely blocked by Fab anti-CD28 monoclonal antibody. At 48 hours post activation, IL-2 secretion seems to be accounted for by B7-1 costimulation, since anti-B7 monoclonal antibody completely inhibits IL-2 production.

Detailed Description Text (174):

In the first round of screening, thirty 100 mm dishes of 50% confluent COS cells were transfected with 0.05 μ g/ml anti-IgM activated human B cells library DNA using the DEAE-Dextran method (Seed et al., Proc. Natl. Acad. Sci. USA, 84:3365 (1987)). The cells were trypsinized and re-plated after 24 hours. After 47 hours, the cells were detached by incubation in PBS/0.5 mM EDTA, pH 7.4/0.02% Na azide at 37.degree. C. for 30 min. The detached cells were treated with 10 μ g/ml/CTLA4Ig and CD28Ig for 45 minutes at 4.degree. C.. Cells were washed and distributed into panning dishes coated with affinity-purified Goat anti-human IgG antibody and allowed to attach at room temperature. After 3 hours, the plates were gently washed twice with PBS/0.5 mM EDTA, pH 7.4/0.02% Na azide, 5% FCS and once with 0.15M NaCl, 0.01 M Hepes, pH 7.4, 5% FCS. Episomal DNA was recovered from the panned cells and transformed into E. coli DH10B/P3. The plasmid DNA was re-introduced into COS cells via spheroplast fusion as described (Seed et al., Proc. Natl. Acad. Sci. USA, 84:3365 (1987)) and the cycle of expression and panning was repeated twice. In the

second and third rounds of selection, after 47 hours, the detached COS cells were first incubated with .alpha.-B7-1 mAbs (133 and B1.1, 10 .mu.g/ml), and COS cells expressing B7-1 were removed by .alpha.-mouse IgG and IgM coated magnetic beads. COS cells were then treated with 10 .mu.g/ml of human CTLA4Ig (hCTLA4Ig) and human CD28Ig (hCD28Ig) and human B7-2 expressing COS cells were selected by panning on dishes with goat anti-human IgG antibody plates. After the third round, plasmid DNA was prepared from individual colonies and transfected into COS cells by the DEAE-Dextran method. Expression of B7-2 on transfected COS cells was analyzed by indirect immunofluorescence with CTLA4Ig.

Detailed Description Text (193):

Human CD28.sup.+ T cells were isolated by immunomagnetic bead depletion using mAbs directed against B cells, natural killer cells, and macrophages as previously described (Gimmi, C. D., Freeman, G. J., Gribben, J. G., Gray, G., Nadler, L. M. (1993) Proc. Natl. Acad. Sci USA 90, 6586-6590). B7-1, B7-2, and vector transfected COS cells were harvested 72 hours after transfection, incubated with 25 .mu.g/ml of mitomycin-C for 1 hour, and then extensively washed. 10.sup.5 CD28.sup.+ T cells were incubated with 1 ng/ml of phorbol myristic acetate (PMA) and 2.times.10.sup.4 COS transfectants. Blocking agents (10 .mu.g/ml) are indicated on the left side of FIG. 12 and include: 1) no monoclonal antibody (no blocking agents), 2) mAb 133 (anti-B7-1 mAb), 3) mAb BB1 (anti-B7-1 and anti-B7-3 mAb), 4) mAb B5 (control IgM mAb), 5) anti-CD28 Fab (mAb 9.3), 6) CTLA-Ig, and 7) control Ig. Panel a of FIG. 12 shows proliferation measured by .sup.3 H-thymidine (1 .mu.Ci) incorporation for the last 12 hours of a 72 hour incubation. FIG. 12, panel b, shows IL-2 production as measured by ELISA (Biosource, CA) using supernatants harvested 24 hours after the initiation of culture.

Detailed Description Text (208):

In the first round of screening, thirty 100 mm dishes of 50% confluent COS cells were transfected with 0.05 .mu.g/ml activated M12 murine B cell library DNA using the DEAE-Dextran method (Seed et al., Proc. Natl. Acad. Sci. USA, 84:3365 (1987)). The cells were trypsinized and re-plated after 24 hours. After 47 hours, the cells were detached by incubation in PBS/0.5 mM EDTA, pH 7.4/0.02% Na azide at 37.degree. C. for 30 min. The detached cells were treated with 10 .mu.g/ml/human CTLA4Ig and murine CD28Ig for 45 minutes at 4.degree. C.. Cells were washed and distributed into panning dishes coated with affinity-purified Goat anti-human IgG antibody and allowed to attach at room temperature. After 3 hours, the plates were gently washed twice with PBS/0.5 mM EDTA, pH 7.4/0.02% Na azide, 5% FCS and once with 0.15M NaCl, 0.01 M Hepes, pH 7.4, 5% FCS. Episomal DNA was recovered from the panned cells and transformed into E. coli DH10B/P3. The plasmid DNA was re-introduced into COS cells via spheroplast fusion as described (Seed et al., Proc. Natl. Acad. Sci. USA, 84:3365 (1987)) and the cycle of expression and panning was repeated twice. In the second and third rounds of selection, after 47 hours, the detached COS cells were first incubated with .alpha.-murine B7-1 mAb (16-10A1, 10 .mu.g/ml), and

Detailed Description Text (209):

COS cells expressing B7-1 were removed by .alpha.-mouse IgG and IgM coated magnetic beads. COS cells were then treated with 10 .mu.g/ml of human CTLA4Ig and murine CD28Ig and murine B7-2 expressing COS cells were selected by panning on dishes coated with goat anti-human IgG antibody. After the third round, plasmid DNA was prepared from individual colonies and transfected into COS cells by the DEAE-Dextran method. Expression of B7-2 on transfected COS cells was analyzed by indirect immunofluorescence with CTLA4Ig.

Detailed Description Text (260):

Supernatants from the hybridomas HA3.1F9, HA5.2B7 and HF2.3D1 were further characterized by competitive ELISA, in which the ability of the monoclonal antibodies to inhibit the binding of biotinylated hCTLA4Ig to immobilized hB7-2 immunoglobulin fusion proteins was examined. Biotinylation of hCTLA4Ig was performed using Pierce Immunopure NHS-LC Biotin (Cat. No. 21335). B7-2 immunoglobulin fusion proteins used were: hB7.2-Ig (full-length hB7-2), hB7.2-VIg (hB7-2 variable domain only) and hB7.2-CIg (B7-2 constant domain only). A hB7.1 -Ig fusion protein was used as a control. For the ELISA, 96 well plates were coated with the Ig fusion protein (50 .mu.l/well of a 20 .mu.g/ml solution) overnight at room temperature. The wells were washed three times with PBS, blocked with 10% fetal bovine serum (FBS), 0.1%

bovine serum albumin (BSA) in PBS for 1 hour at room temperature, and washed again three times with PBS. To each well was added 50 μ l of Bio-hCTLA4-Ig (70 ng/ml) and 50 μ l of competitor monoclonal antibody supernatant. Control antibodies were an anti-B7.1 mAb (EW3.5D12) and the anti-hB7-2 mAb B70 (IgG2b.kappa., obtained from Pharmingen). The wells were washed again and streptavidin-conjugated horse radish peroxidase (from Pierce, Cat. No.